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Briscoe, Kurt G.
Norris McLaughlin & Marcus, PA
875 Third Avenue, 8th Floor
New York, NY 10022

EXAMINER	
SINGH, ANOOP KUMAR	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	**Application No.** 10/685,837	**Applicant(s)** SEIBLER ET AL.	
	Examiner ANOOP SINGH	**Art Unit** 1632	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE _3_ MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on _24 June 2010_.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) _1,5,6,9-12,15-17,20-24,26,27 and 30-38_ is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) _1,5,6,9-12,15-17,20-24,26,27 and 30-38_ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's amendments to the claims filed June 24, 2010 have been entered. Claims 2-4, 7-8, 13-14, 18-19, 25, 28 and 29 have been canceled, while claims 26-27, 37 and 38 have been amended. Claims 1, 5-6, 9-12, 15-17, 20-24, 26-27, 30-37 and 38 are pending in this application.

Election/Restrictions

Applicant's election with traverse of the invention of group IV (27) filed October 24, 2005 was acknowledged. Applicant's argument of examining method for gene knock down in a vertebrate (group 1) with elected group was found persuasive, therefore invention of group I and IV directed to vertebrate and method of gene knock down in a vertebrate were rejoined for the examination purposes. Applicants have also elected SEQ ID NO: 23 as species for claims 31-38. The restriction was deemed proper and therefore made FINAL.

Claims 1, 5-6, 9-12, 15-17, 20-24, 26-27, 30-38 are under current examination.

Priority

It is noted that instant application claims benefit from application number 60/485,969 07/10/2003 that claims benefit of 60/467,814 filed on 05/02/2003, which claims benefit from 60/420,476 filed on 10/22/2002. Upon review of the disclosure of the prior-filed application, '969, '814 and '476 fails to provide descriptive support for instant claims 31-38 generic for elected species of SEQ ID NO: 23. There is not adequate support or enablement for claims 31-35, 37-38 in the manner provided by the first paragraph of 35 U.S.C. 112 in any of these applications. In case, if applicants have evidence to support otherwise, applicants are invited to indicate page and line number for the written support as recited in claims 31-35, 37-38 generic for SEQ ID NO: 23 of the instant application. Therefore, the effective filing date for instant claims 31-35, 37-38 that is generic for SEQ ID NO: 23 is 10/15/2003, while the subject matter of claims 1, 5-6, 9-12, 15-17, 20-24, 26-27, 30 was described in the application no. 60/420476.

Response to arguments

Applicants disagree and argue that claims are supported by provisional application serial No. 60/485,969, filed July 10, 2003, and, therefore, instant claims are entitled to the benefit thereof. Applicants cites page 5-8, example and figure 7 of '969 for the specific support of claims 31-34, 36-38.

Such is found not persuasive because as indicated before that prior-filed application, '969, '814 and '476 fails to provide descriptive support for instant claims 31-35, 37-38 generic for elected species of SEQ ID NO: 23. It should be noted that applicants have previously elected the specie of SEQ ID NO: 23, as specie of shRNA sequence for the generic claim 31 (see applicants' response filed 1/28/2009). There is not adequate support or enablement for claims 31-35, 37-38 in the manner provided by the first paragraph of 35 U.S.C. 112 in any of these applications. It is emphasized that prior filed application fails to disclose the chemical and physical structure of the shRNA sequence (SEQ ID NO: 23) in the claimed method of gene knock down. Therefore, to the extent claims 31-34, 36-38 read on elected specie of SEQ ID NO: 23, the effective filing date for instant claims 31-35, 37-38 remains 10/15/2003.

Withdrawn-Claim Objections

In view of applicants' amendments to the claim 26, the objection to the claim is hereby withdrawn.

Withdrawn-Claim Rejections- 35 USC § 112

Claims 27 and 38 were rejected under rejected under 35 U.S.C. 112, first paragraph, because the specification, because the specification fails to provide an enablement for the full scope of the claimed invention. The previous office action indicated an enabled scope for a mouse having stably integrated an expression vector comprising a shRNA construct under control of a promoter at a polymerase II dependent locus of the mouse genome by homologous recombination, wherein expression of said shRNA results in reduced expression of the gene targeted by said shRNA in said mouse. In view of applicants' amendments to the claims limiting the scope of the claim commensurate with the scope indicated in the previous office action,

instant rejection is hereby withdrawn. Applicants' arguments with respect to the withdrawn rejections are thereby rendered moot.

Maintained-Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 31-35, 37-38 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Lowe et al (US 2008/0226553, dated 9/18/2008; effective filing date: 9/27/2003), Soriano et al (US patent 6,461,864, October 8, 2002, art of record) and Kunath et al., (Nature Biotechnology, 21: 559-561, 2003, IDS) for the reasons of record.

It should be noted that claim 1, 5-6, 9-10, 15-16, 20-24, 26-27 and 30 are not included in the rejection because the claimed subject matter is disclosed in prior provisional application. Further, claims 31-35, 37-38 have been included in the rejection because the effective filing date is 10/15/2003 for the reasons discussed above (see priority section).

Lowe et al teach an expression vector encoding a firefly luciferase shRNA construct flanked by two targeting sequences that target integration of the expression vector to the polymerase II dependent, *hprt* gene locus of a mouse genome. Upon recombination and integration of the expression vector into the *hprt* gene locus, the luciferase shRNA construct is operably linked to the ubiquitous mouse *hprt* promoter (Figure 23). It is also disclosed that the shRNA construct is under control of a ubiquitous promoter as claimed that includes RNA polymerase III U6 snRNA promoter, H1 RNA promoter, tRNA promoter or 7SL RNA promoter (See para. 67-68). Lowe discloses that this expression vector is intended for introduction into mouse embryonic stem (ES) cells (page 17, para, 0172, line 1 to para 0173, line 9). Thus, Lowe clearly discloses all of the limitations of expression vector as claimed. With respect to a method gene knock down, Lowe et al teach providing expression construct comprising shRNA, introducing the luciferase shRNA expression vector, discussed above, into cultured mouse ES cells comprising and expressing a firefly luciferase gene. Lowe further discloses that said introduction of the luciferase shRNA expression vector results in high levels of site specific integration of the expression vector into the *hprt* gene of the mouse ES cells (page 17, para. 0173, line 1-9). Lowe discloses that expression of the luciferase shRNA expression construct by the mouse ES cells effectively suppressed firefly luciferase activity in the ES cells (page 17, para. 0174], lines 1-10). With regard to claim 38, Lowe discloses that the shRNA expression construct and ES cells comprising the shRNA expression construct, as discussed above, are part of a system for creating genetically defined RNAi "epi-alleles" in mice using Cre-mediated

recombination to stably integrate a single RNAi expression cassette into a single locus in the mouse genome. Lowe discloses that this technique will minimize clonal variation due to random integration events. Lowe discloses that the system was developed to mediate the integration of a single shRNA expression cassette into mouse ES cells (page 17, para 0172, lines 1-20). While Lowe et al teach stable integrating the shRNA construct in hprt locus but differ from claimed invention by not disclosing integration of construct into the rosa 26 gene locus.

However, such was known in prior art. For instance, Soriano et al teach methods and vector constructs for the production of genetically engineered non-human animals including mouse, which ubiquitously express a heterologous DNA segment in Rosa 26 locus (abstract and claim 1. col. 9, lines 55-6539). It is noted that Soriano describes targeting region as a portion of a targeting construct which becomes integrated into an endogenous chromosomal location following homologous recombination between a homology clamp and an endogenous gene locus, such as a ROSA26, ROSA5, ROSA23, ROSA11, G3BP (BT5), or EphA2 gene locus sequence (column 3, lines 51-54). Soriano taught a method of targeting region that is flanked on each side by a homology clamp, such that a double-crossover recombination between each of the homology clamps and their corresponding endogenous gene sequences result in replacement of the portion of the endogenous gene locus by the targeting region. However Soriano et al differed from instant method by not disclosing the target sequence being SEQ ID NO: 23 under the control of RNA pol III promoter.

Kunath et al cure the deficiency by teaching a construct comprising DNA encoding the human H1 RNA pol III promoter and a RasGAP shRNA sequence (SEQ ID NO: 23, 100% sequence homology) (see page 561, col. 1, para. 3). Regarding claims 37 and 38, Kunath et al teach a method of gene knockdown by providing the expression vector comprising SEQ ID NO: 23 that are integrated in the genome of ES cells that resulted in inhibition of RasGAP protein (see figure 1 and 2). It is noted that the shRNA disclosed by Kunath et al comprises at least one DNA segment A-B-C wherein A is a 15 to 29 bp DNA sequence with at least 100% complementarily to the gene to be knocked down; B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hairpin molecule, and C is a 19 to 329 bp DNA sequence and further comprises a poly A sequence meeting the limitation of claims (see figure 1A).

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the method of gene knock down disclosed by Lowe et al to include shRNA expression cassettes that are flanked by homology regions for the rosa 26 dependent locus by homologous recombination in ES cells to generate mouse having a single-copy of a transgene inserted at a chosen site in the genome as disclosed by Soriano. The reference of Soriano provided guidance with respect to ubiquitously expressed gene loci to use Rosa 26, rosa5 (see col. 3, lines 49-54). It would have been obvious for one of ordinary skill in the art to further modify the targeting sequence of Lowe by substituting shRNA sequence with another such as one disclosed by Kunath and then flanking by homology regions for the Rosa26 locus to stably integrate expression cassette comprising an shRNA under control of ubiquitous pol III promoter into a specific genomic locus such as HPRT/rosa26 as discussed by Lowe and Soriano with reasonable expectation of achieving predictable result to efficiently suppress the transgene expression. It is noted that several polymerase II dependent loci were known at the time of filing of this application and it would have required only routine experimentation to flank expression cassettes comprising shRNA under the control of a promoter with the homology regions of other

polymerase II dependent locus (See MPEP2144.04). One who would practiced the invention would have had reasonable expectation of success because Lowe provided guidance with respect to produce a mouse comprising an expression vector comprising a shRNA construct that integrates into a polymerase II dependent locus and results in suppression of expression of the gene targeted by said shRNA, while Kunath provided guidance with respect to specific SEQ ID NO: 23. Thus, it would have only required routine experimentation to modify the expression construct disclosed by Kunath that are flanked by homology regions for the polymerase II dependent locus as disclosed by Lowe and Soriano. One of ordinary skill in the art would have combined the teaching of Lowe et al, Soriano and with Kunath because a method of gene knockdown in a mouse comprising a shRNA construct under control of a RNA polymerase III promoter into a specific polymerase II dependent locus that included hprt, rosa26 would have provided stable and sustained expression of short hairpin resulting in gene knockdown.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 1, 5-6, 9-10, 15-16, 20-24, 26-27 and 30 remain rejected under 35 U.S.C. 103(a) as being unpatentable over McCaffrey et al., (Nature, 2002 Vol. 418, 38-39) or Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002) and Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072) for the reasons of record.

Claim 1 is directed to a method comprising (i) stably integrating by homologous recombination an expression vector comprising shRNA construct under the control of a RNA polymerase III promoter and homologous sequence which integrates through homologous recombination at polymerase II dependent locus. It is emphasized that instant rejection is to the breadth of the claims.

McCaffrey et al teach a method of gene knock down in a transgenic mouse comprising an expression vector comprising shRNA under the control of ubiquitous promoter (see abstract). McCaffrey et al teaches delivering an expression vector comprising small-hairpin RNAs (shRNAs) that is expressed *in vivo* from DNA templates using RNA polymerase III promoters inhibiting the luciferase expression by up to 98% (pp38, Figure 1 C-D and pp39 2nd paragraph). McCaffrey teaches shRNA that comprises at least one DNA segment A-B-C wherein A is a 15 to 29 bp DNA sequence with at least 100% complementarily to the gene to be knocked down; B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hairpin molecule, and C is a 19 to 329 bp DNA sequence and further comprises a poly A sequence meeting the limitation of claims 20-24 (see the supplementary information). Although, McCaffrey et al taught a method of gene knockdown in a mouse, but differed from claimed

invention by not disclosing stably integrating the expression construct in a polymerase II dependent locus.

Beach et al disclose that the double-stranded structure may be formed by a single self-complementary RNA strand or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition (pp4, paragraph 52). Beach et al teach the length of the dsRNA is at least 20, 21 or 22 nucleotides in length, e.g., corresponding in size to RNA products produced by Dicer-dependent cleavage. In certain embodiments, the dsRNA construct is at least 25, 50, 100, 200, 300 or 400 bases (pp13, paragraph 16). Beach et al disclose that the dsRNA construct may be synthesized either *in vivo* or *in vitro*. RNA can be derived from an expression construct (pp 13,14; paragraph 168). The invention also discloses strategy for stable expression of dsRNA in cultured mammalian cells (Figure 27, paragraph 78). Beach et al disclose generating several types of short dsRNAs corresponding to the coding region of firefly or Renilla luciferase (pp22; paragraph 246). Beach et al demonstrates that short hairpins encoded on a plasmid are effective in suppressing luciferase gene expression (Figure 42) *in vivo*. DNA oligonucleotide encoding 29 nucleotide hairpins corresponding to firefly luciferase were inserted into a vector containing the U6 promoter. Beach further discloses that one of skill can choose from amongst a range of vectors to either transiently or stably express a short hairpin. Beach et al also disclose non-limiting examples of vectors and strategies to stably express short dsRNAs using U6 and H1 promoters (pp23; paragraph 252; Figures. 43-45). It is noted that Beach et al also disclose that promoters/enhancers that may be used to control the expression of the targeted gene in vivo may include cytomegalovirus (CMV) promoter (see para. 147). Beach et al teach and claim a non-human transgenic vertebrate selected from a list consisting from mouse (see page 12, para. 154) having germline and/or somatic cells comprising a transgene encoding a dsRNA construct (pp 26, claim 28 and pp 2 paragraph 52) that includes rodent (pp12, paragraph 154). Beach et al also demonstrates that a short hairpin is highly effective in specifically suppressing gene expression of firefly or Renilla luciferase (Example 6). However, Beach et al do not explicitly teach how an expression vector integrates through homologous recombination at polymerase II dependent locus.

Prior to instant invention, Bronson describes transgenic mice made by pro nuclear injection of DNA as an effective method of achieving expression of exogenous DNA sequences for many purposes, including over expression, mutant analysis, promoter analysis (see page 9067, column 1, para 1). Bronson also describes problems associated with DNA incorporated into the mouse germ line using this method includes random integration and unpredictable copy numbers. This random integration often also presents profound effect on expression of the transgene resulting in altered phenotype of the mouse (see page 9067, col.1, para. 1). It is noted that Bronson provided advantages of targeting a single copy of a transgenic sequence to a chosen location in the genome such as HPRT over random integration of construct. He discloses many advantages of targeting at specific locus including the ability to control copy number, the ability to insert the transgene into regions of chromatin compatible with a desired developmental and tissue-specific expression. It is noted that Bronson et al emphasize that targeted transgenes provide a more efficient and informative means of securing and comparing the expression of various transgenic sequences than is available with current transgenic procedures. Bronson also

taught homologous recombination in murine ES cells to generate mice having a single-copy of a transgene inserted at a chosen site in the genome (see page figure 2 and page 9068, column 2, para 3). Specifically, Bronson et al disclose a method wherein a single copy murine bcl-2 cDNA driven by either a chicken beta-actin promoter or a human beta-actin promoter has been inserted immediately 5' to the HRPT locus by a directly selectable homologous recombination event (see the abstract and figure 2). However, Bronson et al do not teach expressing shRNA in a specific locus.

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the method of gene knock down disclosed by McCaffrey or Beach to include the shRNA expression cassettes that are flanked by homology regions for the polymerase II dependent locus (HPRT) as disclosed by Bronson to stably integrate by homologous recombination in ES cells to generate nonhuman vertebrate having a single-copy of a transgene inserted at a chosen site in the genome. Bronson provided guidance by emphasizing that the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see page 9072, col. 1, last paragraph). It would have been prima facie obvious for one of ordinary skill in the art to make transgenic nonhuman animal that comprises stably integrated expression vector comprising an shRNA into a specific locus such as HPRT by homologous recombination as discussed by Bronson under the control of polymerase III promoter such as U1 or H1 as disclosed by McCaffrey or Beach in order to more efficiently suppress the transgene expression for sustained period. One who would practiced the invention would have had reasonable expectation of success because McCaffrey/Beach et al had already described a method for gene knockdown in a mice by transiently as well as stably expressing shRNA construct and it would have only required routine experimentation to modify the expression construct that are flanked by homology regions for the polymerase II dependent locus as disclosed by Bronson. One of ordinary skill in the art would have been studied Bronson to combine the teaching of Beach/ McCaffrey because a method of gene knockdown in a mouse comprising a shRNA construct under control of a ubiquitous promoter into a specific polymerase II dependent locus would have provided stable and sustained expression of short hairpin resulting in gene knockdown.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 1, 5, 31-34, 36-38 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002) or McCaffrey et al., (Nature, 2002 Vol. 418, 38-39, art of record); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072, art of record) as applied to claims 1, 5-6, 8-10, 15-16, 18, 20-24, 26-27 above, and further in view of Soriano et al (US patent 6,461,864, October 8, 2002, art of record) for the reasons of record. Claims 31-34, 36-38 are included in the rejection to the extent claims read on the limitation of claim 36 (shRluc and shFluc) and base claim 31 is amended to recite shRluc and shFluc commensurate with the scope of the claim.

The teaching of Beach et al or McCaffrey and Bronson et al have been discussed above and relied in same manner here. Although combination of Beach /McCaffrey and Bronson taught a method of stably integrate by homologous recombination an shRNA construct under the control of a promoter in polymerase II dependent locus (HPRT) but differed from claimed invention by not disclosing stably integrating into other polymerase II dependent locus such as Rosa26.

Soriano et al teach methods and vector constructs for the production of genetically engineered non-human animals, which ubiquitously express a heterologous DNA segment in Rosa 26 locus (abstract and claim 1). It is noted that Soriano describes targeting region as a portion of a targeting construct which becomes integrated into an endogenous chromosomal location following homologous recombination between a homology clamp and an endogenous gene locus, such as a ROSA26, ROSA5, ROSA23, ROSA11, G3BP (BT5), or EphA2 gene locus sequence (column 3, lines 51-54). Soriano taught a method of targeting region that is flanked on each side by a homology clamp, such that a double-crossover recombination between each of the homology clamps and their corresponding endogenous gene sequences result in replacement of the portion of the endogenous gene locus by the targeting region. However Soriano et al differed from instant method by not disclosing using shRNA construct in rosa26 locus.

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the method of gene knock down disclosed by McCaffrey/Beach to include shRNA expression cassettes that are flanked by homology regions for the polymerase II dependent locus (rosa26) by homologous recombination in ES cells to generate nonhuman vertebrate having a single-copy of a transgene inserted at a chosen site in the genome. Bronson provided guidance by emphasizing that the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see supra and page 9072, col. 1, last paragraph). The reference of Soriano provided guidance with respect to ubiquitously expressed gene loci for use include Rosa 26, rosa5 and others (see col. 3, lines 49-54). It would have been obvious for one of ordinary skill in the art to try a method of gene knock down in nonhuman vertebrate by modifying the shRNA expression cassettes under the control of the CMV/H1 or U6 promoter as disclosed by McCaffrey/Beach and then flanking by homology regions for the Rosa26 locus to stably integrate expression cassette comprising an shRNA under control of ubiquitous promoter into a specific locus such as HPRT/rosa26 as discussed by Bronson with reasonable expectation of achieving predictable result to more efficiently suppress the transgene expression. It is noted that several polymerase II dependent loci were known at the time of filing of this application and it would have required only routine experimentation to flank expression cassettes comprising shRNA under the control of a promoter with the homology regions of other polymerase II dependent locus (See MPEP2144.04).One who would practiced the invention would have had reasonable expectation of success because McCaffrey/Beach had already described a method for gene knockdown in a mice by random integration of the construct and it was routine to use express transgene in a chosen site to avoid many of the problems associated with randomly inserted transgenes as evidenced from the teaching of Bronson. Thus, it would have only required routine experimentation to modify the expression construct that are flanked by homology regions for the polymerase II dependent locus as disclosed by Bronson. One of ordinary skill in the art would have been studied Bronson to combine the teaching of Beach/ McCaffrey and Soriano because a method of gene knockdown in a mouse comprising a shRNA

construct under control of a ubiquitous promoter into a specific polymerase II dependent locus that included hprt, rosa26 or any other endogenous loci would have provided stable and sustained expression of short hairpin resulting in gene knockdown.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Claims 11-12, 17 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Beach et al. (US patent Publication no. 2003/0084471, dated 5/1/2003, effective filing date 1/22/2002, art of record) or McCaffrey et al., (Nature, 2002 Vol. 418, 38-39, art of record); Bronson et al (Proc Natl Acad Sci U S A 1996; 93:9067–9072, art of record) and Soriano et al (US patent 6,461,864, October 8, 2002) as applied to claims 1, 5-6, 9-10, 14-16, 20-24, 26-27 and 30 above, and further in view of Ohkawa et al (Hum Gene Ther. 2000; 11 (4): 577-85; IDS) for the reasons of record.

The combined teachings of or McCaffrey/Beach, Bronson and Soriano have been discussed above and are relied upon in same manner. However, none of the reference explicitly teaches an inducible system.

Ohkawa et al teach several constructs composed of the human U6 snRNA promoter and sequences derived from the gene for the tetracycline operator of a prokaryotic tetracycline resistance transposon (abstract). Ohkawa also disclose that expression of the promoter of the human gene for U6 snRNA that contains tet O sequences between the PSE (Figure. 1 and 2) and a TATA box could be be efficiently repressed in cells with the Tet repressor and that this repression can be reversed by tetracycline. Ohkawa et al used this expression system to control the function of an antisense RNA for a fusion gene composed of genes for epidermal growth factor receptor (EGFR) and green fluorescent protein (GFP) and expression of this chimeric gene could be efficiently and rapidly inhibited by tetracycline. However Ohkawa et al do not teach a method to gene knockdown in a nonhuman vertebrate.

It would have been obvious for one of ordinary skill in the art at the time of invention to modify the construct and method disclosed by McCaffrey/Beach to include inducible promoters for shRNA construct wherein operator sequence consist tet as disclosed by Ohkawa. One of ordinary skill in the art would be further motivated to include this construct in a specific locus by homologous recombination in ES cells to generate nonhuman vertebrate having a single-copy of a transgene inserted at a chosen site in the genome wherein transgene could be regulated by tetracyline. Ohkawa provided the provided motivation by showing that tet based system could control the expression of transgene, while Bronson emphasized the use of a chosen site for a single copy of a transgene avoids many of the problems associated with randomly inserted transgenes (see page 9072, col. 1, last paragraph). Furthermore, Bronson and Soriano provided guidance with respect to different endogenous loci including Rosa 26 locus. The person of ordinary skill in the art would have been studied Bronson to make transgenic nonhuman animal comprising stably integrated expression vector comprising an shRNA under the control of ubiquitous promoter into a specific locus such as ROSA26 or HPRT.

One who would practiced the invention would have had reasonable expectation of success because McCaffrey/Beach had already described a method for gene knockdown in a mice by random integration. It would have only required routine experimentation to combine the teaching of McCaffrey/Beach, Bronson, Ohkawa and Soriano because a method of gene knockdown in a mouse comprising a shRNA construct under control of a tet based inducible promoter into a specific ROSA26/HPRT locus would have provided stable and sustained regulated inhibition of transgene.

Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Response to arguments

On page 13, paragraph 1 of the arguments, Applicants disagree with the rejection and point out claims 31-34, 37 and 38 clearly are entitled to the benefit of Provisional Application Serial No. 60/485,969, filed July 10, 2003, which is earlier than Lowe's effective U.S. filing date.

Such is not found persuasive, because of the reasons discussed above (see priority section). It should be noted that applicants have previously elected the specie of SEQ ID NO: 23, as specie of shRNA sequence for the generic claim 31 (see applicants' response filed 1/28/2009). It is emphasized that contrary to applicants' assertion prior-filed application, '969, '814 and '476 fails to provide descriptive support for instant claims 31-35, 37-38 generic for elected shRNA sequence specie of SEQ ID NO: 23. There is not adequate support or enablement for claims 31-35, 37-38 in the manner provided by the first paragraph of 35 U.S.C. 112 in any of these applications. Therefore, to the extent claims 31-35, 36-38 read on elected specie of SEQ ID NO: 23, the effective filing date for instant claims 31-35, 36-38 remains 10/15/2003.

On page 13, paragraph 2 of the arguments, applicants argue that claim 35 is directed to a specific sequence that is not disclosed in prior art.

Such is not found persuasive, because contrary to applicants' assertions Kunath et al teach a RasGAP shRNA sequence that has 100% sequence homology to SEQ ID NO: 23 (see page 561, col. 1, para. 3 and figure 1A). It should be noted that Kunath teaches a construct comprising a RasGAP shRNA sequence (SEQ ID NO: 23, 100% sequence homology) under the control of a human H1 RNA pol III promoter. It is noted that the shRNA disclosed by Kunath et al comprises at least one DNA segment A-B-C wherein A is a 15 to 29 bp DNA sequence with at least 100% complementarily to the gene to be knocked down; B is a spacer DNA sequence

having 5 to 9 bp forming the loop of the expressed RNA hairpin molecule, and C is a 19 to 329 bp DNA sequence and further comprises a poly A sequence meeting the limitation of claims (see figure 1A).

On page 13, paragraph 3-4, Applicants argue that it was not obvious for a person skilled in the art that a single copy shRNA construct under the control of an RNA polymerase III (pol III) dependent promoter can mediate ubiquitous RNA interference in a living organism when integrated into a RNA polymerase II (pol II) dependent locus. Applicants further assert that the cited combination of references, there is no reasonable expectation of success to express a shRNA with an ubiquitously active Pol III construct integrated into a Pol II locus.

Such is not found persuasive, because Applicants have engaged in selective reading of the teachings of Lowe et al. to formulate the grounds for not teaching the invention. In fact, contrary to applicants' assertions Lowe et al teach the combinations of a polymerase II dependent locus and a heterologous polymerase III promoter with a reasonable expectation of success. Lowe et al teach expression vectors comprising polymerase III dependent promoters operably linked to shRNAs. Lowe also teaches targeted integration into a polymerase II dependent locus using hprt flanking sequences around the shRNA construct (supra, para 67-68, 173-175). Lowe further teaches that polymerase III dependent promoters are efficient and effective promoters to drive silencing of a target gene by shRNA. In view of foregoing, it is apparent that Lowe et al , in and of itself, teach all the components of the claims expression vector, cells, and mouse and additionally provides a motivation to produce a variant wherein the shRNA construct has its own promoter, specifically an RNA polymerase III dependent promoter. Kunath et al teach specifically teach a construct comprising a RasGAP shRNA sequence (SEQ ID NO: 23, 100% sequence homology) under the control a ubiquitous RNAase PRNA promoter such as H1 RNA pol III promoter (see page 561, col. 1, para. 3). Regarding claims 37 and 38, Kunath et al teach a method of gene knockdown by providing the expression vector comprising SEQ ID NO: 23 that are integrated in the genome of ES cells that resulted in inhibition of RasGAP protein (see figure 1 and 2). It is noted that the shRNA disclosed by Kunath et al comprises at least one DNA segment A-B-C wherein A is a 15 to 29 bp DNA sequence with at least 100% complementarily to the gene to be knocked down; B is a spacer DNA sequence having 5 to 9 bp forming the loop of the expressed RNA hairpin molecule, and C is a 19 to 329

bp DNA sequence. Therefore, the prior art provides strong motivation to add an RNA polymerase III dependent promoter to the shRNA expression construct of Lowe.

With respect to applicants' argument that there is no reasonable expectation of success in such combinations, it is noted that Lowe et al specifically teach targeted insertion, integration, and expression of an expression vector was successful and predictable in the prior art. Additionally, shRNA expression constructs comprising polymerase III dependent promoters, such as H1 promoter, operably linked to shRNA sequences were well established and predictable in the prior art, as demonstrated by Lowe and Kunath. Therefore, in combination these two elements would have a reasonable expectation of success because the targeted insertion of expression vectors and the expression of shRNA driven by a polymerase III dependent promoter are both well-established and predictable. It should be noted that applicants' have not provided any evidence contrary to the teaching of combination of prior art reference. Furthermore, Lowe et al provide explicit motivation of the use of targeted integration that would minimize clonal variation due to random integration events. Therefore, in view of the fact patterns of the instant case, and the ground of rejection outlined by the examiner, applicants' arguments are not compelling and do not overcome the rejection of record.

Examiner's response to the remaining *three* obviousness rejections are together, as they are all premised basely on the combination of McCaffrey or Beach and Bronson.

Applicants disagree with the rejection and argue that it was not obvious for a person skilled in the art that a single copy shRNA construct under the control of an RNA polymerase III (pol III) dependent promoter can mediate ubiquitous RNA interference in a living organism when integrated into a RNA polymerase II (pol II) dependent locus. On page 15, applicants' argue that McCaffrey and Beach describe a method of gene knockdown in a mouse by administering a shRNA expression vector. However, these references are not instructive in respect to the strategy of targeted integration of a shRNA construct under the control of a pol III dependent promoter into a pol II dependent locus to achieve ubiquitous RNA interference in a living organism. Applicants agree that the reference teaches the mechanism of RNAi mediated gene silencing is functional in mice, the reference not informative in respect to transgenic shRNA expression. Applicants' further argue that Beach demonstrates that a luciferase specific shRNA under the control of the U6 promoter can mediate widespread gene silencing in cultured

cell lines. The teaching of Beach encompasses random integration of shRNA resulting in concatameric array of multiple copies. On page 16, applicants argue Bronson did not provide motivation of targeting a shRNA construct under the control of a pol III dependent promoter into to a pol II dependent locus. Rather, Bronson applied homologous recombination at the HPRT locus to introduce a bcl-2 cDNA under the control of a pol II but not a pol III dependent promoter. The expression level of the targeted bcl-2 transgenes appeared to be non-ubiquitous and varied between the two different constructs. Applicants argue that the activity of a shRNA construct under the control of a pol III dependent promoter as demonstrated by the present invention is neither taught nor suggested by the reference. Applicants further assert that the cited combination of references, there is no reasonable expectation of success to express a shRNA with an ubiquitously active Pol III construct integrated into a Pol II locus.

In response to applicant's arguments against the references individually, one cannot show nonobviousness by attacking references individually where the rejections are based on combinations of references. See *In re Keller*, 642 F.2d 413, 208 USPQ 871 (CCPA 1981); *In re Merck & Co.,* 800 F.2d 1091, 231 USPQ 375 (Fed. Cir. 1986). Applicants' selective reading of McCaffrey/ Beach et al. ignores the teachings of the reference of Bronson et al. There is no requirement for McCaffrey/ Beach et al. to teach that which is clearly taught by Bronson. It would have been obvious to an artisan of ordinary skill to make an expression vector, mouse, and method of gene knock down taught by Bronson, by incorporating a polymerase III dependent promoter into the short hairpin RNA expression construct, taught by Bronson, to drive expression of the short hairpin RNA, as opposed to relying on the polymerase II dependent promoter, using methods known and well established in the art to predictably produce gene knock down in mouse as claimed in the instant application with a reasonable expectations of success. Furthermore, Beach et al provide motivation that teaches that RNA polymerase III dependent promoters provide sire specific initiation and are more effective (see figure 42 and 43). Applicant should further note that prior art taught a construct comprising shRNA under the control of ubiquitous polymerase III promoter as disclosed by McCaffrey/ Beach et al. While the prior art recognized that the local chromosomal environment can affect expression of the integrated transgene and this can result in unpredictable transgene expression as argued by applicant, it is Brosnon et al who provided motivation to integrate construct at a specific genomic

loci by homologous recombination in order to overcome the problem of random transgene integration. This method allows the introduction of single copy transgene into the X-linked hprt locus (see Bronson et al entire article). The art teaches introducing single copy transgene to 5' of the hprt locus by homologous recombination in ES cells. Further the teaching of Bronson also suggests that the hprt locus is a particularly suitable site for the integration of transgene because it exists as an X-linked gene present as a single copy in male ES cells. Moreover, the hprt gene is ubiquitously expressed and so provides a favorable chromatin environment for transgene expression. Bronson et al also show that the level of expression of transgene inserted into the hprt locus is directed solely by exogenous transcriptional regulatory elements (emphasis added) (see page 9071, col. 2, para. 1). To the extent that Brosnon et al. describe the single copy integration of a transgene at hprt locus by homologous recombination to produce stable expression, the rejection in view of McCaffrey/ Beach et al is applicable to the instant case. Thus, the teachings of the cited prior art in the obviousness rejection above provide the requisite teachings and motivations with a clear, reasonable expectation. The cited prior art meets the criteria set forth in both Graham and *KSR*. Therefore, the claimed invention would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention.

Applicants' arguments filed Jne 24, 2010 have been fully considered but are not persuasive. Applicant's arguments all rely on the references of McCaffrey/ Beach, Bronson and Soriano that has been previously discussed. In absence of any other arguments rejection is maintained for the reasons of record.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 1, 5-6, 9-12, 15-17, 20-24, 26-27, 37 and 38 remain provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claim79-82 copending Application No. 11/571194 (20080313747) in view of Kunath et al., (Nature Biotechnology, 21: 559-561, 2003, IDS) for the reasons of record.This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

While Applicant has requested that the rejection be held in abeyance until allowable subject matter can be identified, a request of abeyance does not overcome or address an issue of obvious double patenting between claims 1, 5-6, 9-12, 15-17, 20-24, 26-27, 37 and 38 in the instant case and application 11/571194. Thus, the rejection is maintained.

Conclusion

No Claims allowed.

The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. Gossen et al (Proc Natl Acad Sci U S A. 1992 Jun 15;89(12):5547-51).

Mansour et al., *Proc Natl Acad Sci U S A.* 87(19):7688-92, 1990.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after

the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ANOOP SINGH whose telephone number is (571)272-3306. The examiner can normally be reached on 9:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras can be reached on (571) 272- 4517. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Anoop Singh/
Examiner, Art Unit 1632